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A PRESSURIZED PROPORTIONAL DILUTER FOR AQUATIC TOXICOLOGICAL STUDIES*

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Abstract—A half-liter proportional diluter using positive pressure was designed and tested. Its primary siphons are activated by a pulse of pressure instead of by a partial vacuum as in conventional diluters. This method eliminates the need for venturi tubes, vacuum manifolds, and individual water blocks. Pressure can be supplied by air pumps, air cylinders, or air compressor lines. The diluter's advantages are ease of construction, calibration, compact size, and rapid cleaning. Unit cost is approximately $250.00.

INTRODUCTION

The proportional diluter by Mount & Brungs (1967) is recommended as the best toxicant delivery system for routine use in aquatic toxicity test (Committee on Methods for Toxicity Test With Aquatic Organisms, 1975). The diluter has many advantages over other toxicant delivery systems because it can be constructed from readily available materials, all test concentrations are derived from one stock-solution, test solutions are continuously renewed and are accurate over long periods of time, and the apparatus is relatively trouble-free. Because of these advantages, the proportional diluter is widely used and has undergone several modifications (Esvelt & Connors, 1971; Benoit & Puglisi, 1973; Chandler *et al.*, 1974; Schimmel *et al.*, 1974).

Most diluters in current use employ the venturi vacuum method for dilution operation. In systems such as the Mount & Brungs (1967) proportional diluter, water from a self-starting siphon passes through a venturi tube, producing a partial vacuum which activates certain primary siphons by way of a vacuum manifold. In contrast, the system described in this paper uses a pulse of air pressure to activate primary siphons. As air pressure is applied to the water surface in certain containers, water is forced through the siphon tubes. Pressure is needed only to start the siphons, after which siphoning action takes over.

The system's basic parts are a modification of the diluter described by Schimmel *et al.* (1974). It is designed to deliver six concentrations, a seawater control and a control with carrier at 500 ml per cycle. There is a 50% dilution factor between each concentration. Cycles can be adjusted from 2–6 min in duration. Air pressure can be supplied by portable electric air pumps, compressed gas cylinders, or air compression lines such as found in many laboratories.

* Contribution No. 381 Gulf Breeze Environmental Research Laboratory.

† This project was completed while the author was employed at ERL, Gulf Breeze.

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MATERIALS AND METHODS

Materials

Single-strength, $\frac{1}{8}$ inch window glass was glued together with non-toxic silicon cement to form the head box (*H*), proportioning tiers (*C*,*T*), and mixing boxes (*M*, *FM*) (Fig. 1). The electrical system requires three level switches, two Load Paks (Duval Sensors)®, one normally open three-way soft drink solenoid valve (ASco Red Hat Valves)®, two 110-V receptacles, electrical wire, and (optional) totalizing counter. Other miscellaneous items include: $\frac{3}{4}$ in. marine plywood for backboard and shelves, shelf brackets, $\frac{1}{8}$ in. fiberglass sheeting, neoprene stoppers, and aquarium air tubing. Glass tubing sizes are given in Table 1.

Methods

The positive pressure diluter consists of: (1) the head box; (2) toxicant and carrier mixing boxes; (3) toxicant and carrier proportioning tiers; (4) final mixing boxes (Fig. 1). The positive pressure diluter differs from the diluter described by Schimmel *et al.* (1974) in the following ways: (1) the carrier (*M*-1) and toxicant (*M*-2) mixing boxes are positioned closer together and are on the same level; and (2) the toxicant and carrier tiers are both on the same level, parallel to the backboard and directly below *M*-1 and *M*-2. The carrier tier (*C*-1 to *C*-7) is located behind the toxicant tier (*T*-1 to *T*-7) and both are staggered to facilitate compartment sequence match-up (*T*-2 with *C*-6, *T*-3 with *C*-5, *T*-4 with *C*-4, and so on). The headbox and both toxicant and carrier proportioning tiers are covered with $\frac{1}{8}$ in. fiberglass sheeting. Fiberglass is flexible and the necessary holes can be cut with a key hole saw making its use more convenient than glass.

Holes are cut above the compartments for the placement of siphons, input tubes and/or float switches. The siphons, tubes and switches are held in place by neoprene stoppers and can be adjusted by sliding the siphons or tubes an appropriate distance within the stoppers. The ends of the siphons are flared to facilitate clearing of water from the tubes after siphoning.

Each of the three covered boxes have a pressure/exhaust line of plastic aquarium air tubing. A hole 6 mm in diameter is drilled in each box lid, and a piece of 16 mm (o.d.) acrylic tubing 2 cm long (*E*-2) is glued above the hole. The pressure/exhaust line is held in place by a No. 2 neoprene stopper containing a 6 mm (o.d.) glass tube (*E*-1) which is pressed into the top of the attached acrylic tube. This insures that water will not enter the pressure/exhaust line, and allows for quick disconnection of the container from the line. The ends of the three pressure/exhaust lines are joined together and connected as a unit to the cylinder port of the three-way solenoid valve. The pressure port of

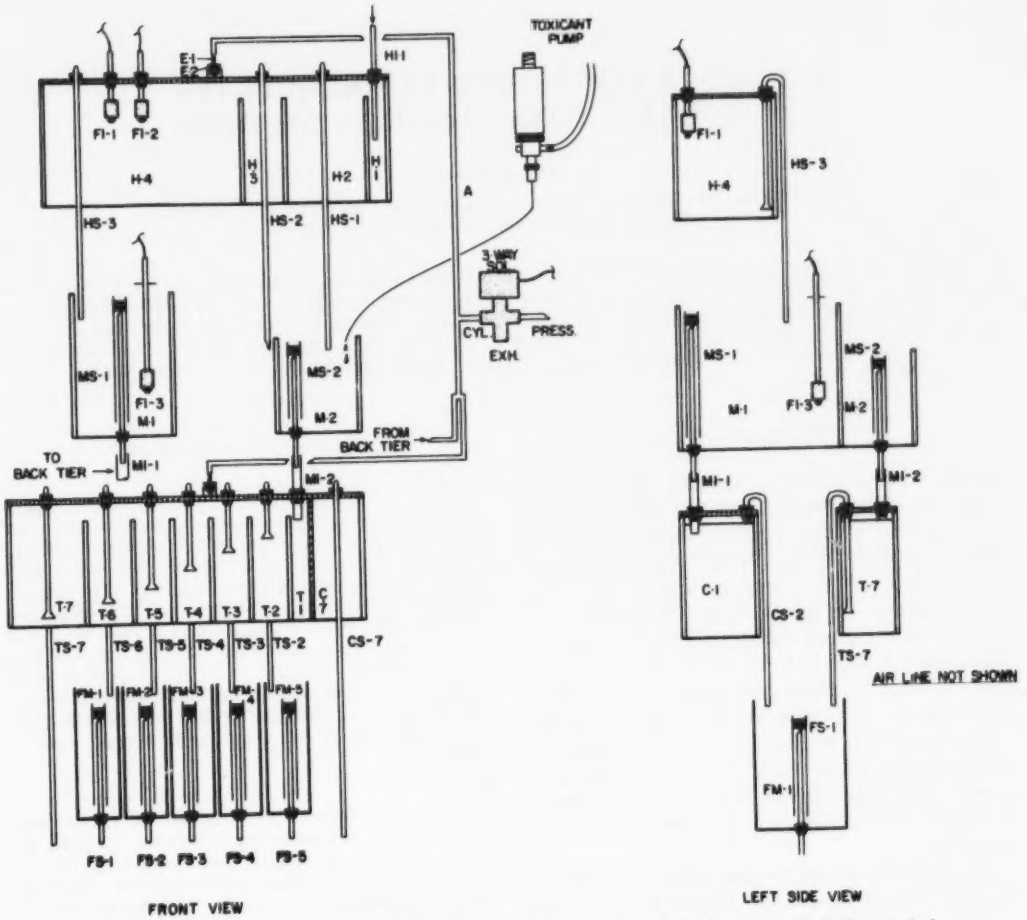

Fig. 1. Detailed schematic drawing of diluter. Legend: *A*-airline; *C*-carrier; *F*-final; *Fl*-float switch; *H*-headbox; *I*-input tube; *M*-mixing box; *S*-siphon; *T*-toxicant.

the solenoid valve is connected to the pressure source; the exhaust port has no connections. Portable electric air pumps, if used, may be plugged into the same circuit as the three-way solenoid (Fig. 3). When the solenoid is energized, the pressure port is open and the pumps are operating; when the solenoid de-energizes, the pressure port closes, the exhaust port opens, and the air pumps are switched off.

The self-starting siphons *MS*-1, *MS*-2, and the final mixing box siphons (*FS*-1 to *FS*-5) are of the design by Benoit & Puglisi (1973). The stand pipes for *MS*-1, *MS*-2 siphons extend into larger diameter input tubes for approximately 2 cm (Table 1). A space must be present between the outer wall of the standpipe and the inner wall of the input tube (Fig. 2). The open space prevents premature starting of siphons due to the rise and fall of small amounts of water in the input tube during tier pressurization and air exhaust.

In the headbox and proportioning tiers, water enters through input tubes that are submerged approximately 1 cm below the container's water level. The input tubes are separated from the remainder of the headbox or proportioning tier by a partition that forms a small compartment. The compartments (*H*-1, *C*-1, *T*-1) allow water to continuously cover the input tubes (*HI*-1, *MI*-1, *MI*-2) so that the input tubes will not act as air exhaust tubes when the containers are pressurized. If an input tube is placed in a compartment containing a siphon, the siphon will not function reliably.

The diluter as described will cycle approximately once every 5 min. The cycle can be shortened by changing the 9 mm siphon in *H*-4 to a 10 mm siphon for faster drainage. Also larger standpipes can be used in *M*-1 and *M*-2 mixing boxes. If *Fl*-3 is moved from *M*-1 to *M*-2, the

Table 1. Tubes used in construction

Tube No.	o.d. (mm)	Material
HI-1	12	Glass
E-1	6	Glass
E-2	16	Plexiglass
HS-1 to 3	9	Glass
MS-1 sleeve	25	Glass
MS-1 standpipe	10	Glass
MS-2 sleeve	22	Glass
MS-2 standpipe	9	Glass
MI-1	20	Glass
MI-2	14	Glass
T-2 to 7	9	Glass
C-2 to 7	9	Glass
FS-1 to 5 sleeve	22	Glass
FS-1 to 5 standpipe	9	Glass

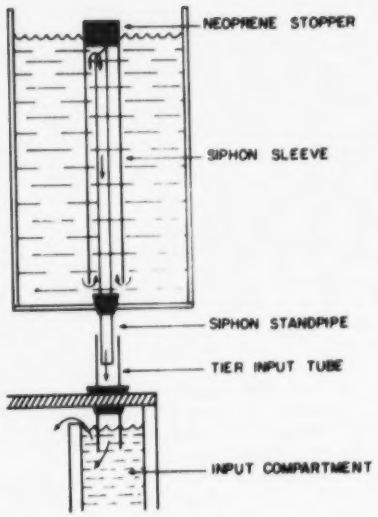

Fig. 2. Mixing box siphon and tier input.

headbox can fill as *M*-1 drains (*M*-2 empties in less time), decreasing cycling time to about once every 2.5 min.

Generally, an equivalent carrier concentration is maintained in all exposure chambers as the toxicant is diluted. In most conventional diluters, equal volumes of carrier without toxicant and carrier with toxicant are injected into carrier and toxicant mixing boxes of equal capacity. This method produces 1531 ml per cycle of excess toxicant water that must be discarded in a conventional half-liter proportional diluter.

The positive pressure diluter eliminates the production of excess toxicant water by using unequal volume carrier and toxicant mixing boxes. The amount of toxicant water produced is equal to the amount needed to fill the toxicant tier. This modification requires the injection of unequal amounts of carrier without toxicant and carrier with toxicant into the appropriate mixing boxes. The amount of water needed to dilute the carrier without toxicant is 2.5 times that needed for carrier with toxicant. The carrier concentration can be equalized in both mixing boxes if the volume injected of carrier without toxicant is three times the volume of carrier with toxicant. The carrier without toxicant is diluted 32% with distilled H_2O to compensate for the discrepancy between injection rate (3.0×) and the necessary rate (2.5×). For example, if 0.025 ml of carrier with toxicant is injected and 0.075 ml of carrier without toxicant (diluted) is injected, the actual amount of carrier without toxicant is 0.051 ml.

PRINCIPLES OF OPERATION

Water enters the headbox by way of compartment *H*-1 from a pump or solenoid valve. The water overflows the partitions and subsequently fills compartments *H*-2 through *H*-4. At this time, the solenoid is de-energized and air displaced by water is forced out through the exhaust lines (*A*) (refer to Fig. 1). A float switch (*Fl*-1) in compartment *H*-4 shuts off the water supply when *H*-4 is filled just below the partition level. Another float switch (*Fl*-2) in *H*-4 is set slightly above that of *Fl*-1. As the water in the headbox levels off in the compartments, *Fl*-2 is tripped and energizes the three-way solenoid; closing the air exhaust port and allowing pressure from the pressure source to enter the headbox and proportioning tiers.

Calibrated amounts of water from the headbox chambers *H*-2 and *H*-4 flow into *M*-2, and *M*-1 mix boxes, respectively, where they mix by turbulence with the injected toxicant and solvent. The float switch (*Fl*-3) in the solvent mix box is in series with *Fl*-1 (Fig. 3) and keeps the input water to the headbox from turning on prematurely. As the water level in *H*-4 lowers, *Fl*-2 drops, de-energizing the three-way solenoid, closing the pressure port, and opening the air exhaust. Chamber *H*-3 is the saltwater control and flows directly to the exposure tank.

At the same time that the headbox is pressurized, both the toxicant and solvent proportioning tiers that were filled on a previous cycle are pressurized. The calibrated toxicant and carrier water from these tiers flow through the siphons into their respective final mixing boxes (*FM*-1 through *FM*-5), mixing and forming the proper dilutions. As the final mixing boxes reach capacity, their self-starting siphons are activated and water of each dilution flows into its appropriate exposure aquarium. The carrier control (*C*-7) and highest concentration (*T*-7) have no mixing boxes and flow directly to the exposure aquaria.

As *M*-1 and *M*-2 reach capacity, the toxicant and carrier tiers are already emptied. The *MS*-1 and *MS*-2 siphons start and their mixtures flow into the proper proportioning tier. The proportioning tiers are now ready for the next pressurization. The float switch *Fl*-3 in *M*-1 drops as *M*-1 is emptied and input water flows again into the headbox. The cycle starts again.

EVALUATION OF PERFORMANCE

Performance and efficiency of the diluter was tested by completing several series of 96-hr acute toxicity tests using the grass shrimp, *Palaemonetes pugio*. Shrimp were exposed to the organochlorine pesticide, endrin, or to the carbamate pesticide, sevin. Duplicate water samples were chemically analyzed for each chemical. Recoveries of these toxicants in seawater were quantitative, i.e. greater than 90%.

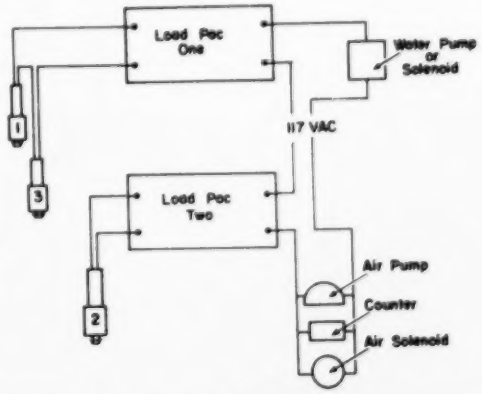

Fig. 3. Electrical system diagram.

SUMMARY

The advantages of a positive pressure method over a vacuum method are: (1) ease in calibration (no vacuum lines are connected to the siphons); (2) elimination of venturi tubes, vacuum manifolds, individual water blocks, and blown glass components; (3) a reduction in space required above and below the diluter for proper functioning of the siphons; (4) the system can easily be disassembled for cleaning.

This positive pressure diluter requires the use of an external mechanical pressure device to operate the apparatus, rather than a self-starting venturi of conventional diluters. However, this diluter design conserves water, toxicant, and carrier, since carrier concentration equalization is achieved with no overflow.

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